3. The stromatal oil of both species was particularly rich in saturated fatty acids. Some of this oil must, therefore, have been tripalmitin which is unusual in fungi.

These quantitative and qualitative differences in the triglyceride oils of sphacelial, sclerotial and stromatal mycelia are not only further indication of the distinctive nature of these tissues, but also raise some interesting speculation concerning fatty-acid metabolism and the factors affecting the changes in metabolism which must occur as sphacelial mycelium differentiates into sclerotial mycelium, and as sclerotial tissues gives rise to stromata. Such processes are thus clearly evident in both the temperate *C. purpurea* and the tropical *C. sulcata.*

REFERENCES

Castagnoli, N. & Mantle, P. G. (1966). Occurrence of D-lysergic acid and 6-methylergol-8-ene-8-carboxylic acid in cultures of *Claviceps purpurea. Nature, London* **211**, 859–860.

Loveless, A. R. & Herd, G. W. (1964). Studies of Rhodesian ergots. 2. Redescription of *Claviceps sulcata* Langdon. *Kirkia* **4**, 41–44.

Morris, L. J. (1968). Fatty acid composition of *Claviceps* species. Occurrence of (+)-theo-9,10-dihydroxystearic acid. *Lipids* **3**, 260–261.

Morris, L. J. & Hall, S. W. (1966). The structure of the glycerides of ergot oils. *Lipids* **1**, 188–196.

Shaw, R. (1966). The polyunsaturated fatty acids of microorganisms. *Advances in Lipid Research* **4**, 107–174.

AN UNUSUAL PARASITIC ASSOCIATION BETWEEN *CLAVICEPS PURPUREA* AND RYE

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Parasitism of the ovary of cereals and grasses by *Claviceps purpurea* (Fr.) Tul. frequently results in the development of the characteristic ergot sclerotium. This fungal structure totally replaces the ovary, the aborted remains of which may be found embedded in the sphacelial tissue which sometimes persists adhering to the distal point of the sclerotium. During experiments which involved the careful dissection of hundreds of rye ears inoculated with *C. purpurea*, several unusual structures comprising both an ergot sclerotium and an apparently normal grain were observed.

A few days before anthesis, florets of field-grown rye ears (Svalof's Fourex Rye) were inoculated by hypodermic syringe with spore suspensions of *C. purpurea* isolates (ex British *Alopecurus myosuroides* ergots, and commercial rye ergot of Portuguese and Polish origin). Eight weeks later the ripe ears were dissected to reveal ergot sclerotia. Six abnormal grain/ergot combinations, some of which were obtained intact, were placed on moist filter paper at 3 °C for 3 days to break dormancy in the embryo, and then transferred to room temperature for 2 days, by which time germination was evident. Some germinated grains were planted in soil and the plants grown to maturity.

One intact grain/ergot combination was fixed in formo-acetic alcohol, embedded in wax using standard histological techniques, and a complete series of longitudinal sections (10 μm) was obtained. The sections were stained with safranin and picro-aniline blue (Vagas & Maacz, 1960) and mounted in euparal.

Plate 41 illustrates the morphological and histological nature of the grain/ergot combinations after germination. All combinations had been attached to the plant at the base of the floret by the proximal end of the ergot sclerotium, and the developing grain had no connexion with any floral component. The grain was structurally typical, although rather smaller than a normal rye grain, having a well-filled endosperm and an intact testa even along the junction with the ergot sclerotium. The germinating embryo had a normal plumule structure and had absorbed part of the endospermal starch through the scutellum. There was no evidence of fungal mycelium within any seed tissue. The medulla and cortex of the sclerotium showed the typical plectenchymatous structure and notably contained no trace of any plant tissue which might have acted as a vascular connexion between the grain and the plant. Neither was there any fungal connexion between the grain and the plant other than the sclerotium.

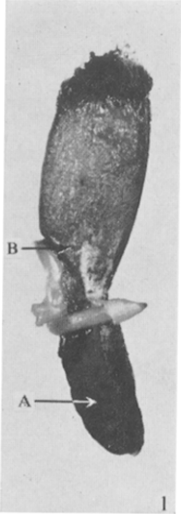
The epidermis of the pericarp was intact except where the embryo had broken through and also where the sclerotium lay adjacent to the testa. Small groups of spores, similar to the sphacelial fructification of *C. purpurea*, were observed on the surface of the distal end of the sclerotium, and similar spores were also found in the sub-epidermal region of the grain.

That normal plants grew from the unusual rye grains confirmed their viability.

This unusual observation raises questions concerning the early stages of the association and the possible function of an ergot sclerotium in translocating adequate nutrients for the development of a viable grain.

It seems clear that infection by *C. purpurea* had occurred near the base of the ovary, but that it had failed to penetrate the inner integument of the ovule, from which the testa is formed, and remained confined to the tissues bounded by the epidermis and the outer integument. At some stage in the development of the ovule the proliferation of sclerotial tissue at its base separated the grain from the plant and left the ruptured epidermis as evidence of the aetiology of the condition. Failure to penetrate the inner integument would have allowed the normal differentiation of embryonic tissues, provided that an adequate supply of nutrient could have been maintained. These nutrients could have been obtained via the fungal tissue either by intra-hyphal translocation or inter-hyphal diffusion. The latter may be less likely owing to the compact plectenchymatous nature of the sclerotium. Under conditions of high relative humidity there might also have been conduction of nutrient over the sclerotial surface.

It is possible that the grain, being green in its early stage of development,



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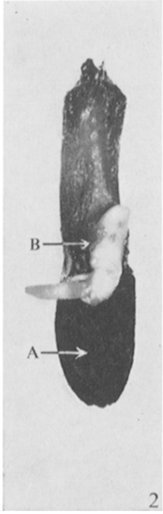

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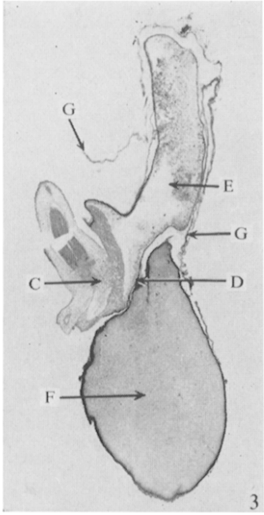

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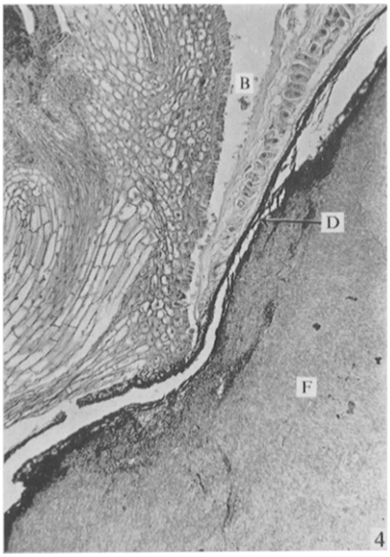

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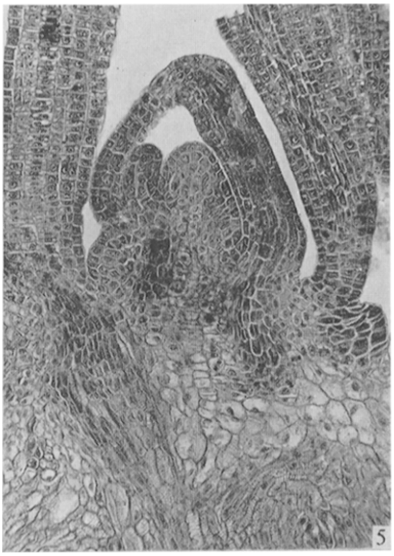

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may have contributed to its own sugar requirement but it seems likely that its photosynthetic capacity would have been insufficient and that some sugar must have been obtained via the fungal tissue. The mean concentration of water-extractable sugars in developing sclerotial tissue has recently been shown to be less than 1 % of the dry weight and to consist principally of fructose and fructosyl polymers (Bassett *et al.* 1972). Thus the developing rye grains may have needed to be able to utilize fructose, which was probably available only in trace amount at the distal end of sclerotium.

Susceptibility of fertilized or unfertilized ovaries to ergot infection has been shown to be lost within, respectively, 9 days after fertilization and 15 days after anthesis (Puranik & Mathre, 1971). Even if, in the present study, the process of infection of a fertilized rye ovary had been delayed until the last possible moment, say, 10 days after fertilization, the ovary would have represented only about 5 % of the normal dry weight of a mature seed. Thus, whether the present unusual structures resulted from late infection or infection at the time of inoculation, at least some of the differentiation and growth of the ovular tissues had occurred after the intercalation of sclerotial tissue between the young grain and the rachilla.

The volume of mature sclerotial tissue was rather less than the average amount which had developed normally in other florets of similarly infected rye ears but it was still within the normal size distribution of ergots. It might be suggested that the sclerotium had only developed, sufficiently to detach the grain from the parent plant, after the grain had reached the observed stage of development, i.e. at least 3 weeks post-anthesis. However, it is considered unlikely that such a regular proliferation of sclerotial tissue would have been delayed to that extent and thus an interpretation involving at least partial development of the grain after detachment from the parent is preferred.

It is recognized that the proposed mechanism for this unusual association between host and parasite is based on circumstantial evidence. Nevertheless, limited infection of the ovary, in which the pathogen fails to penetrate the ovule, does occur and may give rise to structures comprising both seed and ergot sclerotial tissue.

REFERENCES

BASSETT, R. A., CHAIN, E. B., CORBETT, K., DICKERSON, A. G. F. & MANTLE, P. G. (1972). Comparative metabolism of *Claviceps purpurea in vivo* and *in vitro*. *Biochemical Journal* **127**, 3P.

PURANIK, S. B. & MATHRE, D. E. (1971). Biology and control of ergot in male sterile wheat and barley. *Phytopathology* **61**, 1075–1080.

VAGAS, E. & MAACZ, G. J. (1960). A quick method for staining the hyphae of *Ustilago maydis*. *Stain Technology* **35**, 220–222.

EXPLANATION OF PLATE 41

Figs. 1, 2. Two separate ergot (A) germinating grain (B) combinations showing the gross relationship between the attached plant and fungal tissues (× 4·5).

Fig. 3. L.S. through Fig. 2, showing the typical structure of the embryo (C), the intact testa (D) along the junction between the grain and the sclerotium, the part-utilized endosperm (E), the homogeneous nature of the sclerotial tissue (F), which was free from any plant tissue, and the ruptured epidermis (G) of the grain (× 7).

Fig. 4. Detail of the junction between the germinating grain (B) and the sclerotium (F), showing the intact testa (D) and the purity of the plant and fungal tissues (× 30).

Fig. 5. Uninfected plumule apex of the germinating grain has typical arrangement of embryonic tissue (× 75).

RESISTANCE TO BARLEY LOOSE SMUT (*USTILAGO NUDA*) IN THE VARIETY EMIR

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The amount of smut likely to occur in the field can be predicted by embryo examination (Marshall, 1959) and embryo tests are in routine use at Official Seed Testing Stations in the United Kingdom. At Cambridge, 0·2 % embryo infection was found in a sample grown in National Institute of Agricultural Botany (NIAB) variety trials in 1969 and described as Emir. No smutted ears were recorded in this trial or in an earlier one (Doodson, personal communication), which suggested that the variety might show embryo susceptibility but adult plant resistance to the pathogen.

In Canada this type of resistance from the variety Jet was incorporated into the commercial variety Keystone (Johnston & Metcalfe, 1961). One consequence was that routine embryo tests are useless for this variety (Wallen, 1964) and for later varieties with similar resistance. Metcalfe (1966) reported that the Jet gene(s) for resistance to *Ustilago nuda* (Pers.) Rostr. occurred in Emir but made no mention of embryo examination.

In 1969 loose smut was relatively common in England and conditions were not unfavourable for re-infection; samples of Emir barley harvested from plots in S.W. England were examined and three were found to have 0·4, 0·8 and 1·0 % embryo infection respectively. In spring 1970 1000 seeds from each sample were sown in rows at Cambridge; no smutted ears were produced although in other varieties they occurred at levels corresponding to the embryo infection.

During the standard microscope examination (× 20, stereoscopic) of naturally infected Emir samples some infected embryos looked unusual in that the *U. nuda* mycelium appeared coarse or aggregated. Examination under high power showed that the ends of some hyphae were irregularly swollen and balloon-shaped; *U. nuda* mycelium is usually about 3 μm wide but the swollen ends reached 11 μm in width. In June 1970 several ears of Emir were inoculated by the hypodermic needle technique, using an aqueous suspension of spores from a smutted ear of Sultan. Embryo examination of 140 harvested grains showed that 101 embryos contained the fungus.

Three types of resistance to barley loose smut have been recorded: the 'closed-flowering' habit by which florets escape infection (Pedersen, 1960), full physiological resistance to certain races (Popp, 1951) and physiological resistance of the adult plant although embryo infection occurs. In resistant varieties, only for those with the first two types of resistance can embryo